Grant# ES12359

Center Director: Gerald P. Schatten, Ph.D.

Center Overview

Life begins in utero typically. Prenatal environmental exposures, coupled with each zygote's genetics and epigenetic imprints, trace a life history path of health outcomes. The central theme of the Pittsburgh Specialized Center of Research on Sex and Gender Factors Affecting Women's Health is the "Genetic And Environmental Causes Of Adverse Pregnancy Outcomes." This major, but still under investigated, priority for women's health urgently requires multidisciplinary research both for the health of adult women and also for the health of developing fetuses and infants. For women, recurrent spontaneous abortions (RSA) are devastating. We have identified transgenerational transmission of a 'miscarriage gene' that may be an extreme example of deviant genomic imprinting. The implications for fetal outcomes are also of great importance, since in utero development of the fetus, both female and male, establishes the very foundation of the infant, adolescent, and adult. Three research projects along with two research cores and an administrative core are proposed under the directorship of Gerald Schatten, Ph.D. and Sarah Berga, M.D., Clinical Director. Project I, "Pregnancy Loss: Genomic Imprinting and Skewed X-Inactivation" (J. Richard Chaillet, MD, Ph.D., P.1), investigates DNA methylation defects in mice responsible for genomic imprinting as well as skewed X-chromosome inactivation, responsible for RSA in women, Project II, "Epigenetic, Genetic and Environmental Regulation of Pregnancy in Primates" (Gerald Schatten, Ph.D., P.I. and Steve Caritis, MD, Co-P.I.), imaging primate pregnancies and inflammatory responses, addresses sex-specific genomic imprints in genetically controlled and experimentally-manipulated pregnancies. Project III (Julie DeLoia, Ph.D., P.I.), "Maternal and Fetal Consequences of Tobacco Smoke Exposure", analyzes the consequences of smoke exposure in pregnant women and in murine models to understand the interaction of 'genetic variants that jeopardize fetal development and pregnancy. The Imaging Core A performs noninvasive micro-PET and MRI imaging with specific probes, including transgenic MRI/PET reporters. The Pregnancy Core B establishes and maintains pregnancies through conventional and artificial reproductive technologies (ART) in non-human primates and mice. The Administrative Core fosters intra- and inter-SCOR cooperation to facilitate and accelerate basic and clinical research. The multi-disciplinary, interactive, and collegial environments the new Pittsburgh Development Center at Magee-Women's Research Institute right on the contiguous campuses of the University of Pittsburgh and Carnegie-Mellon University, and brings together accomplished teams of clinical and basic investigators inspiring innovations in non-invasive imaging of pregnancy outcomes. Taken together, this comprehensive investigation will answer major women's health problems regarding the dynamic interplay among fetal and maternal genetics, sex-specific genomic imprints and consequences of our first environmental exposures. As such, it is an appropriate and complementary contributor to the ORWH's new SCOR program.

Principal Investigator: J. Richard Chaillet, M.D., Ph.D.

Project 1: Pregnancy Loss:Genomic Imprinting of Skewed X-Inactivation

There is a wealth of information on known or suspected genetic and environmental causes of adverse pregnancy outcomes in women. In addition to these causes, there is now increasing suspicion that defects in epigenetic inheritance are a significant source of many adverse pregnancy outcomes. In this project, the effects of disruptions in normal epigenetic inheritance on pregnancy outcome will be studied, primarily using non-invasive magnetic resonance microscopy (MRM) imaging of in utero mouse fetal development. Germline epigenetic inheritance, or genomic imprinting, is disrupted in a strain of mice carrying a maternal effect mutation in the Dnmt1 (cytosine-5) methyltransferase gene. Heterozygous mutant embryos derived from homozygous mutant females die at different times throughout gestation because of an inability to maintain methylation patterns on imprinted genes. In Specific Aim 1, the developmental course of these heterozygous mutant embryos and their placentas will be assessed by sequential MRM imaging. In Specific Aim 2, diploid-tetraploid chimeric embryos will be produced from wild-type and Dnmtl mutant embryos in order to study the course of fetal development in mice with imprinting defects confined to embryonic or extraembryonic tissues. Compared to the mice studied in Specific Aim 1, these mice will most likely represent a unique spectrum of adverse pregnancy outcomes. In Specific Aim 3, the effects of abnormalities in epigenetic inheritance will be studied by assessing the fetal development of male and female embryos that inherit the recessive X-linked lethal mutations tortoiseshell and bare patches. X-chromosome inactivation will be studied in these mice, as they are potential models for women with skewed X inactivation, which has been recently recognized to be associated with a high incidence of recurrent spontaneous abortion in women. Overall, the goal of this research is to better understand the effects of disruptions in normal germline and somatic epigentic inheritance on fetal development and pregnancy outcome.

Principal Investigator: Gerald P. Schatten, Ph.D.

Project 2: Epigenetic, Genetic and Environmental Regulation of Pregnancy in Primates

Clinical investigations provide insights into the environment and genetic regulation of pregnancy in humans. Furthermore, the wealth of discoveries on non-primates, especially mice, provide solid mechanistic hypothesis that can now be tested using genetic and epigenetic manipulations. This subproject bridges the gap between clinical research and the mouse studies by exclusively focusing on pregnancy in nonhuman primates. In addition to molecular and cell biological investigations, through the use of dynamic and non-invasive MRI and microPET with primates, including ones carrying transgenic reporters, we propose four specific aims. By building on the intellectual foundation of Project I, the first aim studying androgenotes and gynogenotes, answers problems regarding epigenetics and specifically genomic imprinting in primates. The second aim focuses on genetically unmanipulated animals. We will address two important goals. First, we will obtain longitudinal data throughout the gestation of "control" animals in order to better understand the progression of macrophage activation in the "normal" primate pregnancy and labor. Secondly, we will attempt to demonstrate a functional change in normal, full-term parturition as a result of blocking macrophage activation with IL- 10, a powerful anti-inflammatory cytokine. Building on the ability to generate and image successful "normal" pregnancies in Aim II, we will move to Aim III. The third aim addresses pregnancy outcome issues with regards to defined fetal and/or maternal genetics, including identical twins gestated sequentially in the identical surrogate versus identical gestating simultaneously in different surrogates. These studies on the pregnancy establishment and fetal development segue to Aim IV, which examines the inflammatory responses to infectious threats to pregnancy and translates best obstetrical and perinatal clinical care to primate research resource management. The fourth aim investigates the consequences of one of the most important environmental threats to healthy pregnancies - intrauterine bacterial infection. In this aim, we will dynamically image in vivo the maternal and fetal inflammatory response to infection, and also attempt to attenuate that inflammatory response. This aim develops the pregnant primate system and tests the consequences of infection while Project III determines the genetic and epigenetic consequences of smoking during pregnancy in women and in mice.

Principal Investigator: Julie A. DeLoia, Ph.D.

Project 3: Maternal & Fetal Consequences of Tobacco Smoke Exposure

Human pregnancy is a balance between supporting the conceptus and maintaining the health of the mother. Profound cardiovascular and immune system adaptations must occur throughout pregnancy for a successful outcome. The mechanisms and functions of many of these adaptations still allude our understanding; however, we do know that perturbations in both the cardiovascular and immune changes can have deleterious and sometimes devastating consequences to the pregnancy. This proposal will focus on the consequences to pregnancy of one external stress; cigarette smoking. Smoking during pregnancy has many negative outcomes, including an increased risk of early pregnancy loss, premature birth and intrauterine growth restriction. Infants born of mothers who smoked during pregnancy have an increased risk of developing Type 2 diabetes and obesity and suffer from an elevated frequency of chromosomal translocations. The cause and effect relationship between smoking and poor pregnancy outcome most likely occurs at multiple levels. We hypothesize that smoking enhances the inflammatory response that occurs naturally during pregnancy and pushes it out of balance, leading to vascular compromise and fetal and maternal morbidity. We will test our hypothesis by determining to what extent smoking influences the inflammatory response of both mother and fetus, investigate the influence of genetic background on smoking-related consequences and establish a mouse model to examine in utero effects of smoking. Despite concerted public health efforts to reduce cigarette smoking in this country, it remains a leading cause of morbidity and mortality. The prevalence of pregnant women who smoke ranges from 10 - 25%, with consequent long-range health effects to both mother and infant. Successful completion of this proposal should significantly increase our understanding of smoking related morbidity and potentially guide public health professionals to segments of the population that would benefit from more aggressive public hearth intervention strategies.

Principal Investigator: Eric T. Ahrens, M.D.

Core: Imaging

To ensure maximum utility of the Imaging Core, we will combine the strengths of non-invasive magnetic resonance (MR) and positron emission tomography (PET) imaging techniques essential to this SCOR application. At the macroscopic whole animal level, we will utilize new small animal imaging technologies, including MRI, microPET, and microCAT. The primary roles of the Imaging Core will be to: monitor the number of mouse embryos present in pregnancy and measure the size of the embryo and placenta using MRI; perform longitudinal MRI studies of pregnant monkeys and monitor viability and phenotypic differences of the fetus and placenta throughout pregnancy; employ high-resolution 3D MRI of fixed mouse embryos to examine smoking-induced skeletal and vascular alterations; employ non-invasive monitoring of normal pregnancy in non-human primates utilizing PET imaging and glucose and amino acid tracers; delineate paternal vs. maternal contributions in adrogenotes and gynogenotes using reporter gone techniques; to monitor leukocytes invasion and inflammatory response related to the progression of physiologic and pathologic labor and evaluate the effects of smoking on the transport of glucose and amino acids across the placenta. The functions of the Imaging Core will be performed at two locations: MRI methods will be performed at the Pittsburgh NMR Center, Carnegie Mellon University and microPET/microCAT imaging studies will be performed at the UPMC PET Facility, University of Pittsburgh. Both of these facilities are designed for the purpose of providing state of the art non-invasive imaging technologies to biomedical researchers. The MRI component of the Imaging Core will provide a vital resource to the proposed Projects. Projects 1 and 2 will longitudinally follow pregnancies in mouse and in monkey. From these time-lapse data the viability and phenotypic differences of the fetus and placenta will be monitored throughout pregnancy. In Project 3, novel 3D microangiography methods will be utilized to globally examine the impact of smoking on the vascularization of the developing fetus. For the mouse studies, specialized MRI capabilities will be used called magnetic resonance microscopy (MRM); this is an emerging technique capable of imaging biological subjects in vitro and in vivo at near cellular resolution. MRI will help bridge the gap between pregnancy models in mouse and in primates. The Pittsburgh NMR Center has state-of-the art in vivo imaging capabilities for both model systems.

Principal Investigator: Laura Hewitson, M.D.

Core: Pregnancy

The Pregnancy Core (Core B) will provide murine and rhesus gametes, embryos and staged pregnancies (both control and manipulated) to SCOR investigators for investigations on early embryogenesis, imprinting genes critical to implantation, skewed X-inactivation, inflammation during pregnancy loss, smoking during pregnancy, and pregnancy monitoring using non-invasive imaging techniques. The facility provides expertise on ovarian stimulation, in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI), embryo culture, twinning, and embryo transfer for pregnancy establishment, artificial insemination and transgenic approaches for deriving genetically modified offspring. For primates, we principally utilize rhesus monkey oocytes and employ these various techniques to evaluate the fertilizing capacity of sperm, produce embryos at specific stages of preimplantation development and of different genetic reconstitution, and produce transgenic embryos for pregnancy establishment. The Core will provide fertilized rhesus monkey zygotes at the pronuclear stage for transgene infection and cultured embryos for twinning and embryo transfer for pregnancy establishment. Frozen embryos at all stages of preimplantation development are also available to augment the production of fresh material. The Core conducts macaque embryo transfers, both transcervically (non-surgical) and oviductally via laparotomy and establishes timed pregnancies for use in fetal development studies. For mice, we routinely provide gametes, embryos at differing stages, manipulated embryos and timed pregnancies, as well as establishing genetically modified mouse lines. Timed pregnancy establishment for use in non-invasive imaging technologies like MRI and PET analysis (as described in Core A) will also be available for SCOR investigators. Core B provides high quality, cost-effective service for Research Projects I, II and III.